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CLINICAL IMPLICATIONS OF PLASMA FRACTIONATION*

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MANY of the functions of blood are performed by plasma. The fundamental postulate which underlies plasma fractionation is that many functions of plasma are due to the properties of specific plasma components. There are many types of plasma components including such diverse classes of substances as minerals, carbohydrates, and fats. This paper, however, will be limited to a discussion of the consequences of separating the proteins of plasma into preparations which, so far as possible, consist of a single protein.

A detailed consideration of the methods by which plasma fractionation is carried out will not be undertaken, but some reference to the principles involved will be helpful. The most successful plasma fractionation procedure yet devised, and the one which has provided all the commonly used human plasma fractions now available to physicians, is the five-variable system developed by the late Professor Edwin J. Cohn, and his colleagues,[1,2] at Harvard.† This method rests on the assumption

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† The plasma which was used in these studies came from blood collected by the American Red Cross.

that each protein, being a unique component, will under proper conditions, have a different solubility from every other protein. Therefore, each protein could eventually be selectively precipitated and separated from a solution which still contained several other proteins. The variables which define the conditions under which the fractionations occur are the temperature, and the concentrations of ethyl alcohol, of proteins, of hydrogen ions, and of all other small ions such as sodium and chloride.* The description of these protein fractions by numbers, such as I, II and V, refers to the order in which they are precipitated from plasma.

In the past several years Professor Cohn and his associates have been engaged in developing a new method of fractionation.[3-6] This was designed to concentrate and purify plasma proteins in their "state in nature." For this purpose several fundamental modifications of the five-variable system have been introduced. The use of sodium citrate as an anticoagulant when blood is drawn is replaced by an ion exchange resin which removes plasma calcium. The solubilities of the different protein fractions are varied by taking advantage of specific interactions of proteins with metals, such as zinc ions. Consequently, the addition of alcohol and acid buffers, essential parts of the old method, is eliminated. Further, the proteins are fractionally extracted from the relatively stable solid state instead of being fractionally precipitated from solution. This replacement of the sodium citrate, ethyl alcohol and low pH of the old method, which had clearly denatured some proteins and had presumably destroyed others of which little was yet known, by the new reagents and procedures is less likely to alter the characteristics of native proteins.

Table I lists some of the plasma proteins and their physiologic functions. When one considers that the gamma globulins can include innumerable specific antibodies, each of which is a unique protein,[7] that several proteins which play a role in coagulation have been recently discovered, [8, 9] and that there are proteins, like the acid glycoprotein,[10] concerning which little physiologic information is available,[11, 12] it is clear that the eventual number of plasma proteins with which clinicians will be concerned may be closer to a hundred than to the venerable pair which defines the albumin-globulin ratio.

There are at least five advantages to fractionating plasma. Chemical, physiologic and clinical information is obtained; therapy with plasma

* Strictly, the last two variables are not the concentrations of hydrogen ions and small ions but the pH and ionic strength, respectively, which are closely related to concentrations.

TABLE I—PLASMA PROTEINS

Function	*Components*
Oncotic Pressure	Albumins α-, β-, Globulins
Coagulation	Prothrombin Ac-Globulin Antihemophilic Globulins SPCA Precursor Fibrinogen Plasminogen
Immunity	γ-Globulins Complement
Transport	α-, β-, Lipoproteins Transferrin Albumin
Regulation	Hormones
Enzymes	Ceruloplasmin Phosphatases Esterases, etc.

is made more effective by use of plasma derivatives; blood is economically used; the stability of the products obtained is often greater than that of plasma; and greater safety is attained. A few examples will make the advantages clearer.

When a protein is prepared in a relatively pure form by fractionation, the fractionation method itself can be made more effective. This is because the conditions under which the pure protein remains soluble, or is precipitated, may be carefully defined, and its interactions with other compounds may be precisely studied. For example, the fact that the isohemagglutinins of plasma interact specifically with red cell stroma antigens made it possible to devise a method of isolating the isohemagglutinins in solution free of other antibodies.[13]

Equally important is the physiologic knowledge which the chemistry of the protein provides. An example is the insight into iron metabolism gained by an understanding of the interactions of the iron-binding protein of plasma, transferrin, with iron.[14] Conversely, clinical knowledge may stimulate chemical advances. The knowledge of the absence of a coagulation component in hemophilia led to a partly successful search for the specific protein which hemophiliacs lack.[15, 16]

The increased effectiveness of therapy with a plasma derivative is

TABLE II—COMPARISON OF PLASMA PROTEIN FRACTIONS WITH WHOLE PLASMA

Derivative	*Dating Period*	*Clinical Use*	*Max. No. Pts. Treated*	
		20 Blood Donations		
Dried Whole Plasma	5 years	Shock Hypoproteinemia	10	
		Fractionation		
Fraction I	1 yr. +	Afibrinogenemia Postpartum Hemorrhage Hemophilia	11	107
Gamma Globulin	5 yrs. +	Agammaglobulinemia Prophylaxis of infections	90	
Albumin	5 yrs.	Shock Hypoproteinemia	6	

Plus other fractions for research.

emphasized in the case of a plasma protein normally present at a low concentration but yet deficient in a patient. Whole blood or plasma may never be able to supply a sufficient quantity of the protein to overcome the deficiency without seriously overloading the circulation. An example we shall discuss in more detail is the case of fibrinogen in patients with deficiency of this protein.

The economy of blood resulting from fractionation and the stability of the products obtained are exemplified in Table II which has been modified from a table of Janeway's.[17] More than 100 patients can benefit when 5 liters of plasma are fractionated instead of the ten patients who can be treated by this amount of whole plasma. In addition, other products are obtained. Stability of fractionated proteins is at least as great as dried plasma and for some proteins, such as the coagulation components, greater. The conditions for stability are not the same for each plasma protein, and fractionation permits individualization of conditions of storage.

Perhaps the greatest disadvantage to the use of whole plasma, instead of fractions, lies in the ability of pooled plasma to transmit serum hepatitis. Neither attempts to exclude infected donors, irradiation of plasma, nor prophylactic administration of gamma globulin to recipients of plasma have proven fully effective in preventing this disease.[18-22] Fractionation,

however, provides some products, of which albumin and gamma globulin are the most important, which have probably never transmitted the disease in sharp contrast to plasma.[23-26] Newer methods of processing Fraction I, which has transmitted serum hepatitis,[17] seem to have sharply reduced, if not eliminated, the danger of serum hepatitis in patients receiving this product.[27] Thrombin from Fraction III,[28] and proteins from Fraction IV[29] have also transmitted serum hepatitis.

Clinical Use of Plasma Fractions

At present the three principal products of fractionation available to the practitioner for therapeutic use are Fraction I, Fraction II, or gamma globulin, and albumin. None of these products has as yet been prepared in appreciable quantities by the new method of plasma fractionation. In the following discussion we shall deal chiefly with the indications for Fraction I and the gamma globulins, since newly reported clinical entities have enhanced their therapeutic range.

Fraction I, the principal constituent of which is fibrinogen, is supplied as a dry powder. It is generally dissolved in 5 per cent dextrose solution and administered intravenously. There are several therapeutic indications for Fraction I.

Congenital afibrinogenemia is a hemorrhagic disease which is the manifestation of a deficiency of a single plasma protein, fibrinogen.[30] Studies have shown that the defect in these patients is probably an inability to synthesize fibrinogen rather than an increased degradation of the protein.[31] Patients have a clotting time of infinity, that is, no clot forms, and immuno-chemical studies have shown that they possess only about one three-hundredth of the normal amount of fibrinogen. Patients can bleed to death, frequently from umbilical hemorrhage in the early days of life.[30] The administration of one to two grams of Fraction I results in a rise in fibrinogen level, and a fall in clotting time, to normal ranges. This is a rare disease, but its existence and the fact that regular treatment with fibrinogen appears to keep patients perfectly well, have broad implications. There are other congenital diseases which are associated with a specific deficiency of a plasma protein, and, presumably, any one of the plasma proteins could be congenitally absent with clinical consequences dependent on the function of that protein.

Acquired afibrinogenemia can occur as the result of massive transfusions of banked blood since fibrinogen is often destroyed in conven-

tional methods of blood collection and preservation. A very interesting group of obstetrical patients with absent fibrinogen has recently been reported in detail.[32-34] In these women afibrinogenemia can apparently be a consequence of any of three conditions: 1) amniotic fluid infusion, or embolism, in a woman undergoing tumultuous labor; 2) severe premature separation of the placenta; or 3) long-standing intrauterine fetal death. In all three groups intravascular defibrination seems to occur, probably as the result of the introduction of a coagulant from the amniotic fluid or uterine contents into the maternal blood. The syndrome is associated with inability of the maternal blood to form any clot or, if a clot forms, it is at best an unstable one. Severe hemorrhage can occur into the skin and from the gastrointestinal tract as well as from the uterus. There may be some reduction in levels of prothrombin and a fibrinolysin may be demonstrable, but administration of adequate fibrinogen as Fraction I, not as blood transfusions, and appropriate obstetrical care have been successful therapeutically. The amount of fibrinogen that has been used ranged from 2½ to 12 grams with an average dose of about 4 grams.

Fraction I also contains the antihemophilic globulin, a specific plasma protein essential for normal coagulation, which hemophiliacs presumably lack congenitally.[15, 16] Hemophilia is another example of the group of diseases characterized by congenital deficiency of a specific plasma protein. Two to six hundred milligrams of Fraction I, given intravenously, can markedly shorten the clotting time of a hemophiliac patient's blood. Unfortunately, preparations of Fraction I suitable for use in hemophilia must be made from fresh plasma, and, at present, such Fraction I is available only from the Division of Laboratories of the Michigan Department of Health. Fraction I suitable for use in afibrinogenemia is also being prepared by the Michigan laboratories on an experimental basis for the American Red Cross, with limited national distribution.[27]

Fraction II, the gamma globulin of plasma, should be termed gamma globulins since it includes many different antibodies, each of which is a specific protein. It is supplied as a 16.5 per cent solution, and unlike Fraction I and albumin, is always administered intramuscularly, from which site its absorption is prompt. Several indications for its use are known.

Congenital agammaglobulinemia is the third example of the group of diseases of which congenital afibrinogenemia and hemophilia have been

TABLE III—HISTORY OF INFECTIONS IN A BOY WITH AGAMMAGLOBULINEMIA

Age	1	2	3	4	5	6	7	8	9	10	11
Pyoderma	x	x		x			x				
Conjunctivitis		x		x							
Otitis Media		x					x				
Meningitis			x								
Sinusitis		x		x			x x		x		
Pneumonia			xx	x							
Arthritis			x			xx	x x	x			
Sepsis	x										
Viral Diseases			x V	x Mu			x H				
Organism	S	S F	F SF	S P F			F F S S	P			
Blood Culture			F F								
Prophylactic Treatment		NONE →						T'mycin A'mycin		γ-Glob. →	

V=Varicella Mu=Mumps H=Herpes S = Staph. Aur. F=H. Inf. P=Pneum.

mentioned. Patients with this disease have a history of recurrent bacterial infection beginning in infancy, evidence of deficient antibody production and extremely low levels of serum gamma globulins despite essentially normal total proteins.[35-37] The disease may be sex-linked in its inheritance since only males have been observed to be affected congenitally,[37] although the condition may be acquired by children or adults of either sex. Table III, which was prepared by Dr. Leonard Apt of the Children's Medical Center, Boston, Massachusetts, summarizes the course of a boy with agammaglobulinemia. The effect of chemotherapy and of the administration of 0.1 gram of gamma globulin per kilogram of body weight every month, intramuscularly, is shown. Two years have now elapsed since gamma globulin prophylaxis was begun and he has had no infections in that period.

For the prophylaxis and attenuation of infections gamma globulin has found its greatest uses in three virus diseases: measles, infectious hepatitis, and poliomyelitis.[38]

The purpose of using gamma globulin in a healthy child exposed

to measles should be to produce mild measles which will permit permanent active immunity to develop. About 0.02 cc. of gamma globulin solution per pound of body weight will do this if it is given within five days of exposure. Post-measles complications are reduced tenfold by this dose.[24] When complete protection is needed 0.1 cc. per pound given within five days of exposure is effective.[25]

The value of gamma globulin in preventing infectious hepatitis (viral hepatitis A), although not serum hepatitis (viral hepatitis B), has been well shown and recently summarized.[38] Stokes and his collaborators[39] report that as little as 0.01 cc. of gamma globulin solution per pound of body weight may be a protective dose in an institutional outbreak. They suggest that during an epidemic active immunity may develop in susceptible persons given gamma globulin as a consequence of infection occurring while the passive protection conferred by the administered gamma globulin is waning. Another study, in which outbreaks of infectious hepatitis in families were reported, showed, first, that a large percentage of exposed children in a family contracted the disease; second, that gamma globulin is extremely effective in preventing the disease; and third, that adults involved in the outbreaks appear to have a relatively high degree of immunity.[40] It is still uncertain whether the aim in using gamma globulin prophylactically in this disease should be prevention of hepatitis, or attenuation so that active immunity may develop. It is also unsettled whether, despite their high degree of immunity, exposed adults should be given gamma globulin because of the potential danger of the disease in an adult.[40]

The effectiveness of gamma globulin in preventing poliomyelitis in exposed susceptible subjects has been reported by Hammon and his colleagues.[41, 42] Although their conclusions are still not final, gamma globulin appears to confer significant protection against poliomyelitis.

Gamma globulin processed from plasma pooled from the general population, which is the product discussed above, is not effective in the prophylaxis of mumps. When mumps convalescent plasma was used in the preparation of gamma globulin, however, the incidence of mumps orchitis was reduced from 27.4 per cent to 7.8 per cent in adult men given 20 cc. of gamma globulin solution intramuscularly in the first twenty-four hours of the disease.[43]

The available data with respect to the effectiveness of gamma globulin in preventing rubella are too sparse to permit definite conclusions.

Cohn[44] proposed using pooled gamma globulin, drawn from various populations at various times in dry powder form to supply a permanent record, characterizing the immunity of those populations. The information that such gamma globulin records could supply would be of great value in epidemiological investigations. If such material were available, for example, from 1918 to 1919, much light might be shed on the influenza pandemic of those years.

The third plasma fraction generally available for clinical use is albumin, or Fraction V. It is the oldest of the plasma fractions and the need for it in the treatment of wound shock was the chief reason plasma fractionation was undertaken during World War II. It has also been used to replenish protein in hypoproteinemic states and is particularly useful in those which are acute. Its use as a diuretic in such conditions as nephrosis and cirrhosis is wasteful and appears to have no beneficial effect on the underlying disease. Albumin is supplied as a 25 per cent solution and can be prepared in salt-poor solutions containing about a seventh as much sodium as osmotically equivalent plasma.[17] It is administered intravenously and has the great advantage over plasma of safety from the standpoint of homologous serum hepatitis. In the new method of protein fractionation albumin is replaced by "Stable Plasma Protein Solution" which contains all the albumin of plasma plus transferrin, glycoproteins and α-lipoproteins.[5]

I shall not consider the use of albumin in the therapy of shock but re-emphasis of a comment by Janeway concerning albumin as compared to plasma substitutes and extenders seems in order.[17] Although several extenders seem able to substitute for the osmotic activity of albumin, the latter has a fine structure which is responsible for specific binding of many different substances: metals, anions, fatty acids, drugs and organic molecules. The physiologic significance of these interactions is just being comprehended and the use of substitutes which do not possess albumin's unique fine structure and specific properties is an uncertain procedure.

Other Plasma Proteins

I should like now to turn briefly to a consideration of several plasma proteins not available for therapeutic use. The isolation and chemical investigation of these proteins have afforded insight into physiologic and pathologic mechanisms.

Transferrin is the iron-binding protein of plasma,[45, 46] and is present in plasma at a concentration of about 250 mgm. per cent.[14] It has already been discussed at the Fortnight, particularly by Dr. Granick, but it is worth while to mention again the marked specificity of this protein, which can reversibly bind iron and transport it,[14] and, at the same time, abolish the toxic properties which intravascular iron shows when uncombined to this protein.[47] Unlike fibrinogen and gamma globulin no congenital absence or deficiency of this protein has yet been reported. It would be of interest to speculate on what the clinical consequences of "atransferrinemia" might be in the light of chemical knowledge of this protein.

Ceruloplasmin, a plasma protein which contains 0.34 per cent copper, is normally present at a concentration of about 25 mgm. per cent.[48, 49] The bond between the metal and the protein moiety is an irreversible one, unlike that between iron and transferrin.[50] Almost all of the plasma copper is accounted for by ceruloplasmin,[48] which has a deep blue color much more intense than the color of an equal amount of inorganic cupric ion. Ceruloplasmin, which was elegantly crystallized, and characterized by Holmberg and Laurell,[50] is an enzyme which catalyzes the oxidation of amines, phenols and ascorbic acid. A specific deficiency, but not absence, of this protein has been found in patients with Wilson's disease.[49] This is an hereditary, progressive and fatal condition characterized by cirrhosis of the liver, degeneration of the basal ganglia and abnormalities in copper and amino acid metabolism.[51] Patients with this disease possess from 5 to 50 per cent of the normal amount of ceruloplasmin. The concentration of plasma ceruloplasmin has been measured by spectrophotometric and immunochemical methods.[49, 52] Unaffected siblings have normal levels of ceruloplasmin. The level of ceruloplasmin appears to be as low early in the course of Wilson's disease as late, and neither Laennec's cirrhosis[49] nor several other neurologic diseases are associated with deficiency of ceruloplasmin.[53]

It is possible, although not proven, that a deficiency of ceruloplasmin represents the basic defect of Wilson's disease, in analogy with agammaglobulinemia, congenital afibrinogenemia and hemophilia. These would then form a group of four diseases associated with specific deficiences of single plasma proteins. At least four other similar conditions have been described and were mentioned by Dr. Brinkhous: congential hypoprothrombinemia,[54] parahemophilia, or Ac-globulin deficiency,[55, 56] con-

genital deficiency of serum prothrombin conversion accelerator,[57] and Christmas disease or hemophilia B.[58]

It is of interest to consider the possible mechanisms of such deficiencies. Isotopic studies have clearly shown that all the plasma proteins which have been investigated are in the dynamic state, being continually synthesized and destroyed.[59] Decreased synthesis, or increased degradation, or both, could result in deficiency of a plasma protein. As mentioned above, studies on patients with congenital afibrinogenemia suggest that the basic defect is deficient synthesis,[31] and the same appears to be true for agammaglobulinemia.[37] Whether this is the mechanism in all the specific, congenital deficiencies of plasma proteins is still unknown.

Any discussion of deficiency of plasma proteins must take into account the fact that plasma proteins are not confined to the vascular compartment. A dynamic equilibrium exists between intravascular and extravascular plasma proteins.[60, 61] The amount of plasma protein present outside of the vascular compartment is about equal to the quantity in the circulation.[61] This is not only of theoretical importance but of practical significance when administering a plasma protein with the aim of raising its plasma level to a given point. Approximately twice as much protein will be needed as would be calculated from the assumption that the protein will only be distributed in the circulating plasma volume.

A final correlation of disease with plasma protein abnormalities should be mentioned. Patients with atherosclerosis exhibit a changed pattern of plasma lipoproteins. These changes have been elucidated by fractionation in the ultracentrifuge as well as by the chemical methods discussed above.[62-64] The plasma lipids, which include cholesterol, phospholipids and neutral fats, are held in solution in plasma by being bound to two different classes of proteins, the alpha and beta lipoproteins.[65, 66] That these two classes of lipoproteins are quite different compounds is indicated by the fact that the alpha lipoproteins have molecular weights of around 200,000, and a ratio of bound cholesterol to bound phospholipid of about 0.5, while these figures are, respectively, 1,300,000 and about 1.0 for the beta lipoproteins.[63, 65] Table IV, from data of Eder, Russ and Barr, shows how the partition of the total plasma cholesterol between the two classes of lipoproteins differs in subjects with atherosclerosis from the distribution in normal subjects.[67] Cholesterol determinations were done by the Sperry-Schoenheimer method.[68] Patients

TABLE IV—PARTITION OF PLASMA CHOLESTEROL BETWEEN LIPOPROTEINS IN ATHEROSCLEROSIS AND RELATED CONDITIONS

	Total plasma cholesterol	*% in Alpha*	*% in Beta*
Young normal subjects	164	26	74
Atherosclerosis	257	12	88
Nephrosis	503	4	96
Familial hypercholesterolemia	379	9	91

with nephrosis and hypercholesterolemia, conditions in which atherosclerosis is frequently found, show distributions similar to that in atherosclerosis. It is still uncertain whether these changes represent differences from normal in the amounts of the two proteins, in their functional capacity to combine with lipids or to a defect in lipid metabolism which these changes reflect. The description of these variations in lipoproteins in these diseases constitutes an important advance toward understanding the nature of atherosclerosis.

An attempt has been made in this paper to show that plasma fractionation is bound closely to clinical medicine. Fractionation has made therapeutic protein preparations available which possess a considerable number of advantages over the parent plasma from which they are derived. There is every likelihood that the number of these preparations will increase as research proceeds. Plasma fractionation has also afforded insight into physiology and disease on a chemical level by unravelling many of the functions of plasma and ascribing some of these to purified, often single, proteins. As a result, therapy with blood has become more rational, relations between single plasma proteins and disease have appeared, and several diseases have been described which indicate that congenital deficiencies of single plasma proteins may be responsible for a considerable number of hereditary illnesses.

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REFERENCES

1. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M. and Taylor, H. L. Preparation and properties of serum and plasma proteins; a system for the separation into fractions of the protein and lipo-

protein components of biological tissues and fluids, *J. Amer. chem. Soc. 68*:459-75, 1946.

2. Strong, L. E. Blood fractionation, in *Encyclopedia of chemical technology,* edited by R. E. Kirk and D. F. Othmer, New York, Interscience Encyclopedia, Inc., 1948, v. *2,* pp. 557-83.
3. Cohn, E. J. Characterization and separation of proteins by virtue of their interactions, in *Blood cells and plasma proteins; their state in nature,* edited by James L. Tullis, New York, Academic Press Inc., 1953, pp. 18-58.
4. Cohn, E. J. Blood collection and preservation, *Proc. Amer. Assoc. Blood Banks 4*:34-37, 1951.
5. Surgenor, D. M. Recent advances in the preparation of stable plasma derivatives, *Quart. Rev. Med. 9*:145-52, 1952. (In *Int. Rec. Med. 165,* August 1952).
6. Tullis, J. L. Newer technics for the separation and fractionation of whole blood, *N. Y. St. J. Med. 53*:525-27, 1953.
7. Enders, J. F. The concentrations of certain antibodies in globulin fractions derived from human blood plasma, *J. clin. Invest. 23*:510-30, 1944.
8. Ware, A. G. and Seegers, W. H. Plasma accelerator globulin; partial purification, quantitative determination, and properties, *J. biol. Chem. 172*:699-705, 1948.
9. Alexander, B., Goldstein, R. and Landwehr, G. Prothrombin conversion accelerator of serum (SPCA); its partial purification and its properties compared with serum Ac-globulin, *J. clin. Invest. 29*:881-95, 1950.
10. Schmid, K. Preparation and properties of serum and plasma proteins; separation from human plasma of polysaccharides, peptides and proteins of low molecular weight; crystallization of an acid glycoprotein, *J. Amer. chem. Soc. 75*:60-68, 1953.
11. Howe, C. The influenza virus receptor and blood group antigens of human erythrocyte stroma, *J. Immunol. 66*:9-35, 1951.
12. Highberger, J. H., Gross, J. and Schmitt, F. O. Interaction of mucoprotein with soluble collagen; an electron microscope study, *Proc. nat. Acad. Sci. 37*:286-91, 1951.
13. Isliker, H. C. Purification of antibodies by means of antigens linked to ion exchange resins, *Ann. N. Y. Acad. Sci. 57*:225-38, 1953.
14. Laurell, C.-B. What is the function of transferrin in plasma? *Blood 6*:183-87, 1951.
15. Taylor, F. H. L., Davidson, C. S., Tagnon, H. J., Adams, M. A., Macdonald, A. H. and Minot, G. R. Studies in blood coagulation; the coagulation properties of certain globulin fractions of normal human serum in vitro, *J. clin. Invest. 24*:698-703, 1945.
16. Minot, G. R., Davidson, C. S., Lewis, J. H., Tagnon, H. J. and Taylor, F. H. L. The coagulation defect in hemophilia; the effect in hemophilia of the parenteral administration of a fraction of the plasma globulins rich in fibrinogen, *J. clin. Invest. 24*:704-07, 1945.
17. Janeway, C. A. Clinical use of blood derivatives, *J. Amer. med. Assoc. 138:* 859-65, 1948.
18. Rosenthal, N., Bassen, F. A. and Michael, S. R. Probable transmission of viral hepatitis by ultraviolet-irradiated plasma, *J. Amer. med. Assoc. 144:* 224-26, 1950.
19. Barnett, R. N., Fox, R. A. and Snavely, J. G. Hepatitis following the use of irradiated human plasma, *J. Amer. med. Assoc. 144*:226-28, 1950.
20. James, G., Korns, R. F. and Wright, A. W. Homologous serum jaundice associated with use of irradiated plasma, *J. Amer. med. Assoc. 144*:228-29, 1950.
21. Duncan, G. G., Christian, H. A., Stokes, J., Jr., Rexer, W. F., Nicholson, J. T. and Edgar, A. Evaluation of immune serum globulin as a prophylactic agent against homologous serum hepatitis, *Amer. J. med. Sci. 213*:53-57, 1947.
22. Stokes, J., Jr., Blanchard, M., Neefe, J. R., Gellis, S. S. and Wade, G. R. Methods of protection against homologous serum hepatitis; studies on the protective value of gamma globulin in homologous serum hepatitis SH virus, *J. Amer. med. Assoc. 138*:336-41, 1948.
23. Paine, R. S. and Janeway, C. A. Human

albumin infusions and homologous serum jaundice, *J. Amer. med. Assoc. 150*:199-202, 1952.

24. Janeway, C. A. Use of concentrated human serum gamma globulin in prevention and attenuation of measles, *Bull. N. Y. Acad. Med. 21*:202-22, 1945.
25. Ordman, C. W., Jennings, C. G., Jr. and Janeway, C. A. The use of concentrated normal human serum gamma globulin (human immune serum globulin) in the prevention and attenuation of measles, *J. clin. Invest. 23*:541-49, 1944.
26. Greenstein, N. M., Barenberg, L. H. and Steinberg, D. Relation of homologous serum hepatitis to prophylactic injection of human blood fractions; study of 13,755 cases with injections, *Amer. J. Dis. Child. 81*:218-25, 1951.
27. Surgenor, D. M. Personal communication to the author.
28. Porter, J. E., Shapiro, M., Maltby, G. L., Drake, M. E., Barondess, J. A., Bashe, W. J., Jr., Stokes, J., Jr., Oliphant, J. W., Diefenbach, W. C. L., Murray, R. and Leone, N. C. Human thrombin as a vehicle of infection in homologous serum hepatitis, *J. Amer. med. Assoc. 153*:17-19, 1953.
29. Hsia, D. Y., Kennell, J. H. and Gellis, S. S. Homologous serum-hepatitis following the use of Fraction IV prepared from postpartum plasma, *Amer. J. med. Sci. 226*:261-64, 1953.
30. Henderson, J. L., Donaldson, G. M. M. and Scarborough, H. Congenital afibrinogenaemia; report of a case with review of the literature, *Quart. J. Med. 14*:101-11, 1945.
31. Gitlin, D. and Borges, W. H. Studies on the metabolism of fibrinogen in two patients with congenital afibrinogenemia, *Blood 8*:679-86, 1953.
32. Reid, D. E., Weiner, A. E. and Roby, C. C. Intravascular clotting and afibrinogenemia; the presumptive lethal factors in the syndrome of amniotic fluid embolism, *Amer. J. Obstet. Gynec. 66*: 465-74, 1953.
33. Weiner, A. E., Reid, D. E. and Roby, C. C. Incoagulable blood in severe premature separation of the placenta; a method of management, *Amer. J. Obstet. Gynec. 66*:475-99, 1953.
34. Reid, D. E., Weiner, A. E., Roby, C. C. and Diamond, L. K. Maternal afibrinogenemia associated with long-standing intrauterine fetal death, *Amer. J. Obstet Gynec. 66*:500-06, 1953.
35. Bruton, O. C. Agammaglobulinemia, *Pediatrics 9*:722-28, 1952.
36. Bruton, O. C., Apt, L., Gitlin, D. and Janeway, C. A. Absence of serum gamma globulins, *A. M. A. Amer. J. Dis. Child. 84*:632-36, 1952.
37. Janeway, C. A., Apt, L. and Gitlin, D. Agammaglobulinemia, *Trans. Assoc. Amer. Phycns. 66*:200-02, 1953.
38. Barondess, J. A. Present status of gamma globulin, *Pediatrics 10*:732-42, 1952.
39. Stokes, J., Jr., Farquhar, J. A., Drake, M. E., Capps, R. B., Ward, C. S., Jr. and Kitts, A. W. Infectious hepatitis; length of protection by immune serum globulin (gamma globulin) during epidemics, *J. Amer. med. Assoc. 147*:714-19, 1951.
40. Brooks, B. F., Hsia, D. Y. and Gellis, S. S. Family outbreaks of infectious hepatitis; prophylactic use of gamma globulin, *New Engl. J. Med. 249*:58-61, 1953.
41. Hammon, W. M., Coriell, L. L. and Stokes, J., Jr. Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis; plan of controlled field tests and results of 1951 pilot study in Utah, *J. Amer. med. Assoc. 150*:739-49, 1952; and Conduct and early follow-up of 1952 Texas and Iowa-Nebraska studies, *Ibid. 150*:750-56, 1952.
42. Hammon, W. M., Coriell, L. L., Wehrle, P. F., Klimt, C. R. and Stokes, J., Jr. Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis; preliminary report of results based on clinical diagnoses, *J. Amer. med. Assoc. 150*:757-60, 1952.
43. Gellis, S. S., McGuiness, A. C. and Peters, M. Study on the prevention of mumps orchitis by gamma globulin, *Amer. J. med. Sci. 210*:661-64, 1945.
44. Cohn, E. J. Blood and blood deriva-

tives, *Amer. Scient. 33*:61-83, 1945.
45. Schade, A. L. and Caroline, L. An iron-binding component in human blood plasma, *Science 104*:340-41, 1946.
46. Koechlin, B. A. Preparation and properties of serum and plasma proteins; the beta$_1$-metal-combining protein of human plasma, *J. Amer. chem. Soc. 74:* 2649-53, 1952.
47. Cartwright, G. E. and Wintrobe, M. M. The anemia of infection; studies on the iron-binding capacity of serum, *J. clin. Invest. 28*:86-98, 1949.
48. Holmberg, C. G. and Laurell, C.-B. Investigations in serum copper; isolation of the copper containing protein and a description of some of its properties, *Acta. chem. scand. 2*:550-56, 1948.
49. Scheinberg, I. H. and Gitlin, D. Deficiency of ceruloplasmin in patients with hepatolenticular degeneration (Wilson's disease), *Science 116*:484-85, 1952.
50. Holmberg, C. G. and Laurell, C.-B. Investigations in serum copper; coeruloplasmin as an enzyme, *Acta. chem. scand. 5*:476-80, 1951.
51. Denny-Brown, D. and Porter, H. Effect of BAL (2, 3-dimercaptopropanol) on hepatolenticular degeneration (Wilson's disease), *New Engl. J. Med. 245:* 917-25, 1951.
52. Scheinberg, I. H. and Gitlin, D. *J. clin. Invest.*, in press.
53. Scheinberg, I. H. *J. clin. Invest.*, in press.
54. Rhoads, J. E. and Fitz-Hugh, T., Jr. Idiopathic hypoprothrombinemia — an apparently unrecorded condition, *Amer. J. med. Sci. 202*:662-70, 1941.
55. Owren, P. A. Parahemophilia; hæmorrhagic diathesis due to absence of a previously unknown clotting factor, *Lancet 1*:446-48, 1947.
56. Frank, E., Bilhan, N. and Ekren, H. Die Parahämophilie (Owren), eine neue Form der hämorrhagischen Diathese, *Acta haemat. 3*:70-90, 1950.
57. Alexander, B., Goldstein, R., Landwehr, G. and Cook, C. D. Congenital SPCA deficiency; hitherto unrecognized coagulation defect with hemorrhage rectified by serum and serum fractions, *J. clin. Invest. 30*:596-608, 1951.
58. Soulier, J. P. and Larrieu, M. J. Differentiation of hemophilia into two groups; a study of thirty-three cases, *New Engl. J. Med. 249*:547-53, 1953.
59. London, I. M. Studies on the rates of formation of serum proteins in man, in *Symposia on nutrition,* vol. 2, *Plasma proteins,* Springfield, Ill., Charles C. Thomas, 1950, pp. 72-82.
60. Whipple, G. H. and Madden, S. C. Hemoglobin, plasma protein and cell protein, *Medicine 23*:215-24, 1944.
61. Gitlin, D. and Janeway, C. A. Dynamic equilibrium between circulating and extravascular plasma proteins, *Science 118*:301-02, 1953.
62. Lindgren, F. T., Elliott, H. A. and Gofman, J. W. Ultracentrifugal characterization and isolation of human blood lipids and lipoproteins with applications to the study of atherosclerosis, *J. phys. Colloid Chem. 55*:80-93, 1951.
63. Russ, E. M., Eder, H. A. and Barr, D. P. Protein-lipid relationships in human plasma; in normal individuals, *Amer. J. Med. 11*:468-79, 1951.
64. Barr, D. P., Russ, E. M. and Eder, H. A. Protein-lipid relationships in human plasma; in atherosclerosis and related conditions, *Amer. J. Med. 11*:480-93, 1951.
65. Oncley, J. L. and Gurd, F. R. N. The lipoproteins of human plasma, in *Blood cells and plasma proteins; their state in nature,* edited by James L. Tullis, New York Academic Press Inc., 1953, pp. 337-57.
66. Eder, H. A. and Russ, E. M. Composition and distribution of plasma lipoproteins in normal and pathological states (abstract), *J. clin. Invest. 31:* 626, 1952.
67. Eder, H. A. Personal communication to the author.
68. Sperry, W. M. and Webb, M. Revision of the Schoenheimer-Sperry method for cholesterol determination, *J. biol. Chem. 187*:97-106, 1950.